Transformation of Plasmids:

If DNA is on a piece of filter paper cut it out and put into a test tube with 100 ul water/TE (10mM tris, 0.1mM EDTA)

Locate or buy some competent E-coli. We use Nova blue (In-vitrogen), XL-blue (stragagene form the stockstore), or topo (from TA cloning kit, also from In-vitrogen). They are stored at –80 in 20 ul aliquots

Add 1 ul of DNA to one tube of E-coli. Controls are: no DNA or control plasmid DNA (like PUC, Bluescript or similar)

Incubate cells and DNA 30 minutes on ice.

Heat shock ~45-90 seconds at 41 C

Add 100 ul of "SOC" (from stock, a yellow nutrient media) and shake at 37 C (to recover)

Plate 10 ul and 90 ul separately in LB (luria broth) agar plates containing 50ug/ml ampicillin and incubate the plate upside down 37C overnight

Select colonies for amplification in LB broth containing amp (stock is located –20C, dilute 1/1000, ie for 500 ml media use 500 ul).

Extract DNA from overnight cultures using a mini (5 ml) midi (50 ml) or maxi (500ml) plasmid preparation kit. Before spinning cells (as per the protocol in the kit) save 700 ul and add 300 ul 50% glycerol in a nunc tube. Save this stock at –80 for future use.

Optional: squirt Xgal (50 ul of 10% in n-n dimethlyformamide to dissolve) and 50 ul IPTG (0.1 M in water) for "blue white selection" onto the plates and gently rub in with a flamed bent glass rod.

Determine if the DNA you transformed is correct by:

1) Studying the restriction map of the clone and select diagnostic restriction enzymes to test. Run a 1% agarose gel and stain with ethidium bromide.
2) PCR the DNA using universal primers that flank the insertion site (multipurpose cloning site) using restriction enzyme digest.
3) Send plasmid to Nikki for DNA sequencing using primers adjacent to the cloning site and compare the returned sequence by "blast" analysis (http://www.ncbi.nlm.nih.gov/gorf/bl2.html).